Sex Determination: Two Copies for One Cock

The molecular mechanism of sex determination in birds has long remained mysterious. Genetically male chicken embryos, which have two Z sex chromosomes, develop female gonads when the Z chromosome-linked gene *DMRT1* is knocked out. This suggests that sex is determined by Z chromosome dosage.

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Most people would know that it takes a Y chromosome to make a human male. Biology students would also know that such a dominant role of a sex-limited chromosome is not the only way sex chromosomes may determine sex. In fruit flies and the nematode *Caenorhabditis elegans*, for instance, it is the dose (number) of X chromosomes that is critical to sex determination. However, when it comes to birds — the sister lineage of mammals — where males have two copies of the Z chromosome and females one copy of Z and one copy of the W chromosome, even experts have remained puzzled about how sex is determined [1]. Now, in a recent paper, evidence is presented that the Z-linked gene *DMRT1* determines male sex in chicken, possibly by a dosage effect [2].

There have been two main hypotheses for the genetic basis of avian sex determination: either, the female-specific W chromosome contains a dominant factor necessary to trigger female development (analogous to the Y-linked *SRY* gene triggering male development in mammals); or, the dose of Z chromosomes governs whether a bird develops a male or a female phenotype. The cytogenetic characteristics of the Z and W sex chromosomes have not helped much to distinguish between these hypotheses. Much like the sex chromosomes of other groups, the Z and W chromosomes of birds are derived from a normal pair of autosomes that gradually stopped recombining [3] after acquiring a role in sex-determination. Similar to most Y chromosomes, the W chromosome of extant birds is usually small, mainly consists of heterochromatin and is filled with repeat arrays. So far, only a few W-linked genes have been identified in the main avian model, the domestic chicken.

One possible candidate for avian sex determination is the gene for the doublesex and mab-3 related transcription factor 1 (*DMRT1)*. This gene is known to be a key regulator of sexual development across a wide range of organisms, such as worms, flies and fish [4–6]. In humans, it is obviously not the sex-determining gene, yet its critical role in sex determination is indicated by the fact that hemizygosity of the autosomal human *DMRT1* gene (due to a chromosomal deletion) leads to deficiency of testicular development and XY feminization [7]. Such haploinsufficiency clearly shows the importance of having two functional copies of *DMRT1* for male development in a mammal. When it was found that *DMRT1* is located on the Z chromosome in chicken and other birds [8], the idea emerged that *DMRT1* could be the sex-determining gene because of its different expression levels in males and females, which have two and one copies of the gene, respectively.

In a recent paper, Smith and colleagues [2] used RNA interference to knock out the *DMRT1* gene in chicken. Two retroviral vectors containing either microRNA or short hairpin RNA targeted against *DMRT1* were delivered into developing embryos and sexual development was monitored. When *DMRT1* was knocked out, embryos with two Z chromosomes, thus genetically male, developed gonads that both macroscopically and histologically resembled those of normal females, that is, a left-side ovary and a right-side regressing gonad (in female birds, the ovary develops only from one side). These feminized birds also showed significantly reduced expression of *Sox9*, which is normally up-regulated

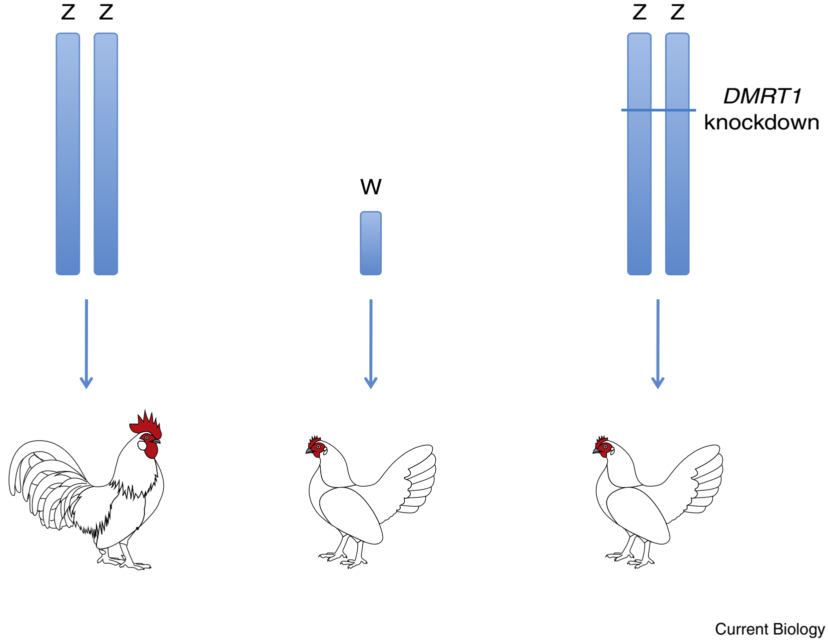

Figure 1. Scenarios for the role of sex chromosomes in bird sex determination.

There are two principal possibilities for how sex could be chromosomally determined in birds. First, the presence of two Z chromosomes (containing a male-determining factor) leads to male development (left panel). As females, which chromosomally are ZW, also have one Z chromosome, this requires the putative male-determining factor to be dosage sensitive. Second, the presence of a single W chromosome leads to female development (middle panel). This posits a female-specific gene on the W chromosome, much like the Y-liked male determinant SRY in mammals. Right panel: knockout of *DMRT1* leads to female development in ZZ chickens, lending support for a role of Z chromosome dosage.

in male embryos, but increased expression of the female-specific marker gene *aromatase*. However, when *DMRT1* was knocked out in genetically female (ZW) embryos, normal female development ensued (Figure 1).

Do these findings then settle the debate on whether avian sex determination is a matter of Z chromosome dosage or a dominant role of the W chromosome? While the evidence for *DMRT1* playing a critical role in the initiation of male development seems solid, it remains possible that the W chromosome contains a similarly critical factor for female development. Specifically, it has been hypothesized that in female birds such a W-linked factor could block the function of a male-determining locus on the Z chromosome. One candidate for such a factor has been the W-linked gene *HINTW* — one of very few W-linked genes that is functionally different from its paralogous copy on the Z chromosome, *HINTZ* [9]. An attractive hypothesis posits that HINTW functions as a heterodimer with HINTZ, possibly by interacting with a male hypermethylated (*MHM*) region located adjacent to the *DMRT1* locus [10,11]. However, it was recently found that genetically male (ZZ) chickens is mis-expressing *HINTW* still develop normal testes [12]. It thus remains to be seen if the avian W chromosome contains a dominant ovary-determining factor, and if so, what it is.

If Z chromosome dose is indeed critical to avian sex determination by virtue of the expression level of *DMRT1* in developing ZZ and ZW embryos, it is necessary that the difference in gene copy number translates into a corresponding difference in the amount of protein. In other words, the expression of *DMRT1* should not be dosage compensated, unlike the majority of sex-linked genes in mammals and in most other organisms with sex chromosomes. However, it was recently found that chickens, and probably other birds, lack sex chromosome dosage compensation altogether [13,14]. This observation was unexpected, but — given the new data on *DMRT1* and male development — one might speculate that the absence of dosage compensation is perhaps somehow related to the dose-dependent role of *DMRT1* in sex determination.

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